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# ICMSTEA 2014: RECENT RESEARCH AND ISSUES ON MATHEMATICS, SCIENCE, TECHNOLOGY, EDUCATION AND THEIR APPLICATIONS

# ISOLATION AND STRUCTURE ELUCIDATION OF B-SITOSTEROL COMPOUND FROM THE N-HEXANE FRACTION OF ROOT WOOD OF PTEROSPERMUM SUBPELTATUM C.B. ROB

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## Abstract

and structure elucidation of β-sitosterol compound has been performed for the first time from -hexane extracts of Pterospermum subpeltatum C.B. Rob (Sterculiaceae) wood root. The wood powder of P. subpeltatum was extracted with methanol, and the methanol extracts was partitioned -hexane. N-hexane fraction showed activity against brine shrimp (Artemiasalina) with LC50 each 44,02; 76,68; 396,42; > 500 ppm. The molecular structure of compound is determined based on interpretation of the data of IR spectroscopy, 1H and 13 C-NMR.

**Keywords:** Pterospermum subpeltatum, β-sitosterol, Artemiasalina.

## Introduction

Pterospermum plants including one of Sterculiaceae family genus and grows Primary forests or grown locally in secondary forests and on the edge of the river on soils to grow at an altitude of 1400 sea level. Pterospermum consists species found in India, Burma Indo-China, South China, and the entire region of Malaysia Papua New Guinea, as well as on islands in Indonesia (Boer *et al*, Pterospermum commonly known as and have different names based on the growth areas, such as in Java as walang and Sumatra by the name koras. In Sulawesi, this plant is found several areas, among others Production Bantaeng (banjoro), a screen at Muna (rumbei). spermum plants are included in the aceae family widely been used as medicine for the people of Sulawesi (Kruit *et el* in Heyne, *P. javanicum* bark is used to cure ery, toothache, boils, and sprains. *P.diversifolium* leaves drug known as it ching and root bark of this plant is used as a fish poison (Ogata *et al*, 1995). Camporese *et al* 2003 reported on the antibacterial lactivityof N-hexane extract and methanol extract of stem bark of *Guazumaulmifolia* (Sterculiaceae). N-hexane extract can inhibit the growth of *E. coli*, while the methano lextract inhibited the growth of *Pseudomanasae ruginosa*. Reid *et al.* (2005) studied the antibacterial activity of various extracts of *Cola green way* and reported that the ethyl acetate fraction actively inhibit the growth of *Klebsiellapneu moniae* and *Staphylococcus aureus*. Search phylogenetic several plants of Sterculiaceae species, steroid compounds found them stigmesterol glucoside and ethyl acetate extract of *Ambromaaugusta* roots (Alam *et al*, 1995). Our literature shows no studies reporting about the bioactivity of compoundsof 5majorfraction of N-hexane and compounds β-sitosterol from *Pterospermum subpeltatum* C. B. Robrootwood. Molecular structure of the compound is determined based on the interpretation of data covering

specteroscopy of IR, NMR ($^1$H,$^{13}$C, HMQC, HMBC and H-H COSY)

The melting point is determined by means of John Fisher, infrared spectra obtained with a Perkin Elmer FTIR spectrophotometer, while1 H (500 MHz) and 13 C (125MHz) NMRJ eol determined with a spectrophotometer.

## 2. Material and Method

### 2.1. Material

Plant material used is root wood *Pterospermum subpeltatum* C.B. Rob, obtained from the district Produktion West Sulawesi and has been determined in the Bogor-based, Center for Research and Development Biology, LIPI Bogor.

### 2.2. Method

The method used is the extraction and isolation. A total of 10kg of dry weight of root wood *Pterospermum subpeltatum* C.B. Rob pulverized and then macerated with methanol for 1x24 hours several times. Macerat obtained is evaporated until a viscous macerat obtained by 3L with 1,047 kg dry weight. The macerat further partitioned with N-hexane solvent. N-hexane fraction (43.53 g) was fractionated by vacuum columnchromatography (VCC) with eluent N-hexane, ethyl asetatin-exane, ethyl acetate, acetone and methanol with increasing polarity which produces five main fractions. The third fraction from the KVC (12.7g) was further separated by press column chromatography (PCC) with the same fluent as above KKV process isolates obtained yellowish white. Furthermore, the crystallization with a solvent of chloroform and methanol to obtain shiny white crystals obtained by weighing120 mg were then re-crystallized with hot methanol yield as much as 79 mg of white crystals (compound X).

**Biological test.** Toxicity tests performed using fry shrimp *Artemia salina* in accordance with the method of Meye *al.*

## 3. Results and Discussion

CompoundX obtained as a wh powdert .l. 130-131 °C: IR (KBr) νmax 3412, 2956, 2935, 2866, 1664, 1462, 1049,28 cm-1; 1H NMR (500 M $CDCl_3$); and$^{13}$C-NMR (125 MHz, CDC shown in Table 1.

Compound X was obtained as a wh needle crystal clear, t.l. 130-13 Spectrum IR (KBr) shown absorption ba at wave number 3412 $cm^{-1}$, identify presence of free OH, in 2956, 2935 a 2866 $cm^{-1}$ for C-H aliphatic,1664 $cm^{-1}$ ou group (C=C), 1462 $cm^{-1}$($CH_2$), and 1 $cm^{-1}$ ($CH_3$)and in 1049,28 $cm^{-1}$for stretch vibration C-O. Spectrum $^1$H-N compound (1) showed a signal at the 1,12; 1,85; 2,27; 2,01; 1,48; 1,98; 1,56 a 1,63 ppm each (2H, m) asmethylene sig atδ: 3,52; 1,48; 0,93; 1,05; and 1,08 ppm each (1H, m) and in 5,34 ppm (1H, t, J= Hz) which indicates the presence methyline.At theδ 0,67 and 1,00 ppm a (3H, s, H-18 and H-19) as the methyl sig Free OH group is indicated by the signa δ 5,10ppm (brs). Proton signals, indica the presence of the steroid skele substituted by two methyl and hydroxyl. In the aliphatic region seem some signals that indicate a unit of alip is 3 signals for a methylene group δ (2H, m, H-22), 1,15 and 1,25, th methylene at theδ 1,35, 0,91 dan 1,66 p (1H, m, H-25) and 4 methyl group at t 0,92 (3H, d, J=6,7 Hz, H-21), 0,81, 0,8 0,85 ppm, ten proton signal is a framework. Data Analysis spectrosco $^{13}$C-NMR showed 29 carbon signals wh represent 29, which includes a carbon on δ 71,9 ppm and 2 carbon alkene δ 140,9 and 121,9. Spectrum of DEPT show positive carbon signals consisting carbon methyl on δ : (12,0; 12,1; 18,9;

Table 1. Spectroscopic Data of $^{1}$H-NMR and $^{13}$C-NMR X Compound

| $^{1}$H-NMR δ ppm (H, multiplicity, konstkopling ) | $^{13}$C-NMR δ ppm | HMBC (H→ C) | COSY (H →H) | β-Sitosterol (Holland, 1978 |
|---|---|---|---|---|
| 1,84 (2H, m) | 37,4 | C-4 | - | 37,3 |
| 1,85 (2H, m) | 31,8 | C-3, C-10 | 3 | 31,8 |
| 3,52 (1H, m) 5,10 (brs, 3-OH) | 71,9 | - | 4 | 71,9 |
| 2,27 (2H, m) | 42,4 | C-5,C-6, C-3, C-10 | - | 42,4 |
| - | 140,9 | - | - | 140,9 |
| 5,34 (1H, t, J=4,9Hz) | 121,9 | C-4, C-8, C-10 | 7 | 121,8 |
| 2,0 (2H, m) | 32,09 | - | - | 32,0 |
| 1,48 (1H, m) | 32,06 | - | - | 32,0 |
| 0,93 (1H,m) | 50,3 | - | 11 | 50,3 |
| - | 36,6 | - | - | 36,6 |
| 1.49 (2H, m) | 21,2 | - | - | 21,1 |
| 1,98 (2H, m) | 39,9 | C-14, C-9 | - | 39,9 |
| - | 42,4 | - | - | 42,4 |
| 1,05 (1H, m) | 56,9 | - | - | 56,8 |
| 1,56 (2H,m) | 24,4 | - | - | 24,3 |
| 1,63 (2H, m) | 28,4 | - | - | 28,2 |
| 1,08 (1H, m) | 56,2 | - | 20 | 56,2 |
| 0,67 (3H, s) | 12,0 | - | - | 11,9 |
| 1,0 (3H, s) | 19,5 | C-5, C-9, C-10 | - | 19,8 |
| 1,35 (1H, m) | 36,3 | - | - | 36,2 |
| 0,92 (3H, d, J=6,7 Hz) | 18,9 | - | - | 18,8 |
| 1,03 (2H, m) | 34,0 | C-21 | - | 34,0 |
| 1,06 (1H,m) | 29,3 | | 24 | 29,3 |
| 0,99 (1H, m) | 45,9 | - | - | 50,3 |
| 1,25 (1H, m) | 26,2 | C-29 | 27 | 26,2 |

| Position | $^1$H-NMR δ ppm (H, multiplicity, konstkopling ) | $^{13}$C-NMR δ ppm | HMBC (H→ C) | COSY (H →H) | β-Si (Holl |
|---|---|---|---|---|---|
| 26 | 0,81 (3H, m) | 19,1 | - | - | |
| 27 | 0,83 (3H, m) | 20,0 | C-28 | - | |
| 28 | 1,25 (2H, m) | 23,2 | - | 29 | |
| 29 | 0,85 (3H,m) | 12,1 | - | - | |

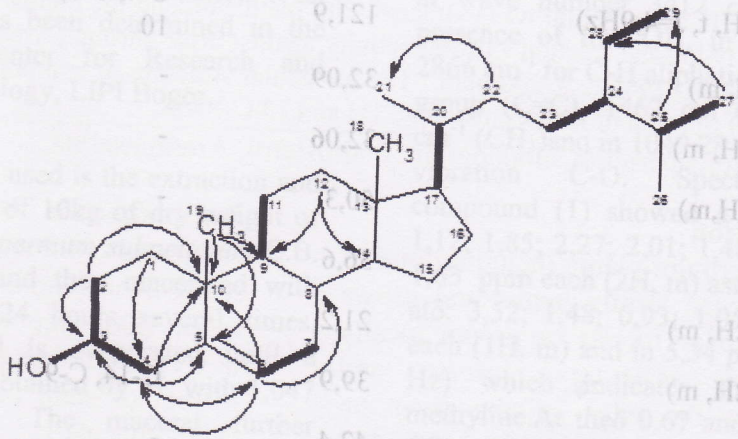


Figure 1.

19,5; and 20,0 ppm), 9 carbon methylen on δ: (71,9; 121,9; 32,06; 50,3; 56,9; 56,2; 36,3; 45,9 and 26,2 ppm). This Spectrum of DEPT carbon also shows the negative signal of the methylene carbon 11δ : 37,4; 31,8; 42,4; 32,09; 21,2; 39,9; 24,4; 28,4; 34,0; 29,3 and 23,2 ppm. From these data it can be seen the presence of quaternary carbon are: δ: 140,9; 36,6 and 42,4 ppm . Twenty-nine of the carbon signals from the skeleton of steroids with a double bond at C-5 and C-6 atoms. C-17 of the steroid skeleton bound to the C-20 atom from alkanes unit. From the data correlation of HMBC (Table 1) between (1H) and (13C) proton, it can be concluded that compound X is β-sitosterol as shown Figure 1.

The above data were also compa with data from the literature as shown Table 1 on this comparison is to ensure the compound X is β-sitosterol. In the of ($LC_{50}$) againts *A. salina* of the fractions of N-hexane A1-A5 are 40,02, 76,68, 396,42 ppm and >500 non-toxic. This was showed that the hexane fraction largely active *A. Salina.*

## Conclusion

β-sitosterol compound have been isolated from the main fraction of the N-hexane fraction A3 which has a toxicity value ($LC_{50}$) 76,68 ppm.

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## References

Achmad, S.A. 2007. Keaneka Ragaman Hayati Dalam Pembelajaran Ilmu Kimia. Prosiding Seminar Nasional Jurusan Kimia Universitas Negeri Makassar.

Alam, M.S., Chopra, N., Ali, M. and Niwa, M. 1995. Oleanen and Stigmasterol Derivatives from *Ambromaaugusta. Phytochemistry*, 41 (4) 1197-1200.

Boer, E., Lemmens, R.H.M.J. 1998. Plant Resources of South-East Asia: Timber trees: Lesser-known timbers No. 5 (3). Bogor Indonesia.

Camporese, A., Balick, M. J. F., Arvigo, R., Esposito, R. G., Marsellino, N., De Simone, F., Tubaro, A., 2003. Screening of anti-bacterial medicinal plants from Belize (Central America). *Journal of Ethnopharmacology*, **87,** 103-107.

Cragg, G.M., D.J. 2002. Drugs from nature, present developments and future prospects. Dalam Rauter, A.P. dkk (Ed), *Nature Products in the New Millennium Prospects and Industrial Application.* 285-297.

Harborne, J.B. 1984. Metode Fitokimiadan Penuntun Cara Modern Menganalisis Tumbuhan. ITB Bandung.

7. Heyne, K. 1987. Tumbuhan Berguna Indonesia III. Badan Litbang *Kehutanan, Jakarta.*
8. Hatano, T., Miyatake, H., Natsume, M., Osakabe, N., Takizawa, T,. Ito, H., Yoshida, T. 2002. Proanthacyanidinglycocides and related polyphenols from cacao liquor and their antioksidant effects. *Phytochemistry*. **59**. 749-758
9. Holland, H.L., Diakow, P.R.P., Taylor, G, J., 1978, Can. J. Chem. **56**. 3121-3127.
10. Manitto. 1981. *Biosintesis of Natural Products,* John Willy and Son., New York
11. Martens, S., and Mithofer, A. 2005. Molecules of Interest Flavones and Flavone Synthesis. *Phitochemistry*. **66**. 2399-3407
12. Meselhy, M.R. 2003. Constituents from moghat, the Roots of *Glossostemonbruguieri*Deft. *Molecules*. **8.** 614-621
13. Meyer, B.N., Ferrigny, N.R., Putnam, J.E., Jacobbsen, L.B., Nicols, D.E., Mc Laughlin, J.L. 1982. Brine Shrimp, A. Covenient General Bioassay for Active Plant Contituent. *Medical Plant Research* .**45**. 31-34
14. Ogata, Y.(Committe Members). 1995. *Indeks Tumbuh-tumbuhan Obat di Indonesia*. PT. Esai Indonesia. Edisi II
15. Reid, K. A. Jager, A. K., Light, M. E., Mulholland, D. A., Van Standen, J. 2005. Phytochemical and pharmalogical screening of sterculiaceace spesies and isolation and antibacterial compounds. *Journal of Ethnophamacology*, **97**, 285-291.
16. Rocha, J.C.B., Pedrochi, F., Hernandes, L., Mello, J.C.P., Baesso, M.L. 2007. Ex vivo evaluation the percutaneous penetration of proanthocyanidin extracts from *Guazumaulmifolia* using

photoacoustic spectroscopy. *Analytica ChimicaActa.* **587**. 132-136.

17. Soekarno, N.H., Achmad, S.A., Ghisalberti., Aimi, N., Hakim, E.H., dan Syah, Y.M. 2003b. Artoindonesianin X dan Y, two new Isoprenilated 2-arilbenzophurans, from *Artocarpus fretessi* Hassk. *Phytochemistry.* **64**. 831-834.
18. Segawa, P.S., Kasenene, J.M. 2007. Medicinal plant diversity and uses in the Sango by area Southern Uganda. *Ethnopharmacologi.* **113** 521-540
19. Shrestha, P.M., Dhillion, S.S. 2003. Tradisional medicinal plant use and diversity in the highlands of Dolakha District Nepal. *Ethnopharmacologi.* **86.** 81-89.

photoacoustic spectroscopy. *AnalyticaChimicaActa*.**587**. 132-136.

Soekarno, N.H., Achmad, S.A., Ghisalberti., Aimi, N., Hakim, E.H., dan Syah, Y.M. 2003b. Artoindonesianin X dan Y, two new Isoprenilated 2-arilbenzophurans, from *Artocarpus champessi* Hassk. *Phytochemistry*. **64**. 831-834.

Tegegrawa, P.S., Kasenene, J.M. 2007. Medicinal plant diversity and uses in the Sango by area Southern Uganda. *Ethnopharmacologi*.**113** 521-540

Tamang, P.M., Dhillion, S.S. 2003. Traditional medicinal plant use and diversity in the highlands of Dolakha District Nepal. *Ethnopharmacologi*. **86.** 87-89.